Electronic Supplementary Material (ESI) for RSC Advances.

Cryostorage of unstable N-acetylglucosaminyltransferase-V by synthetic zwitterions

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Contents

1. Materials and Methods

1.1. Cell culture

COS7 cells were purchased from RIKEN Cell bank and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum at 37°C under 5% (v/v) CO_2 condition.

1.2. Purification of recombinant GnT-V

COS7 cells at approximately 50% confluency grown on 15-cm dishes were transfected with plasmid expressing a soluble form of human GnT-V (Thr121 to Leu741)[1] using Polyethylenimine (PEI) Max (Polysciences, 24765) reagent according to the manufacturer's protocol. After 4 h, the culture medium were replaced with Opti-MEM I (ThermoFisher Scientific) and cells were further cultured for 72 h. His-tagged recombinant GnT-V was purified from the cell culture medium by affinity chromatography using Ni^{2+}-Sepharose 6 Fast Flow (GE Healthcare), followed by a purification by size exclusion chromatography using NAP-5 gel filtration column (GE Healthcare) with 1 mL of elution buffer [50 mM 2-Morpholinoethanesulfonic acid (MES; pH 6.2)].

1.3. In vitro enzyme activity assay of GnT-V before and after freezing

Activity of GnT-V was measured as described previously[2]. Purified GnT-V solution with or without zwitterions were prepared by mixing GnT-V solution (48 µL) with MilliQ (12 µl) or glycerol (12 µL) for controls or 25% or 50% of each of zwitterion (12 µl) for 5% or 10% zwitterion solutions, respectively. For determination of activity before freezing, these GnT-V solutions (2 µl) were incubated in 10 µL of GnT-V reaction buffer [125 mM MES (pH 6.25), 10 mM EDTA, 200 mM GlcNAc, 0.5% Triton X-100, and 1 mg/ml BSA] supplemented with 20 mM UDP-GlcNAc and 10 µM acceptor *N*-glycan substrate [GnGnbi-PA (PA, 2-aminopyridine)] at 37°C for 30 min, followed by incubation at 99°C for 2 min to inactivate the enzyme. For determination of activity after freezing, residual GnT-V solutions were stored at -80°C overnight, followed by thawing at room temperature, and enzyme reactions were performed as described above. For determination of retention time of GnT-V product and the non-specific peaks from zwitterions, control reactions without GnT-V solutions were performed under the same reaction conditions. After enzyme reactions, the samples were diluted with MilliQ (40 mL) and analysed by separation of the product from the unreacted substrate by reverse-phase HPLC. Activity was calculated based on the peak areas of the products and unreacted substrates.

The ZIs were synthesized as previously reported.[3, 4] The glycerol solutions were prepared with the concentrations based on vol% using pipettes as typical biologists do. On the other hand, the ZIs were difficult to prepare at volume/volume because they are solid. The ZIs were thus prepared at wt% in the present study.

1.4. Determination of protein amount

Purified GnT-V dissolved in solutions with or without zwitterions prepared in 1.3. section was separated by SDS-polyacrylamide gel electrophoresis. GnT-V protein was stained and visualized by Coomassie Brilliant Blue staining using Gel Code Blue (ThermoFisher Scientific). The amount of GnT-V protein before and after freezing was calculated from their band intensities and the standard curve generated by band intensities of bovine serum albumin.

1.5. FTIR spectral measurement

The amide I' spectra (1600~1690 cm^{-1}), which arise from the mainly C=O stretching vibration in the deuterated peptide group, is highly sensitive to changes in the secondary structure of proteins[5]. Since the second derivative analysis showing the negative peaks is widely used to investigate the change in

each secondary structure of the proteins[6], we used this analysis to investigate the detail structural change of bovine α-lactalbumin (Sigma-Aldrich Co.) in 0%, 5% and 10% OE_2imC_3C solution before/after freezing. FTIR spectra of α-lactalbumin (10 mg/ml) were recorded using a Nicolet 6700 FTIR spectrometer (Themofischer Scientific. Co.) equipped with an MCT liquid nitrogen detector. Each spectrum was obtained by co-adding 512 scans at a spectral resolution of 4.0 cm^{-1}. Deuterium oxide (D_2O) (99 %, Kanto Chemical Co.) was used instead of H_2O because the absorption of the amide I band of proteins overlaps that of the bending band of H_2O. 30 μL sample solutions were sealed in a transmittance-type cell (MagCell) consisting of CaF_2 windows separated by a 10 μm Teflon spacer (Jasco). The spectra of solvent solution (0%, 5% and 10% OE_2imC_3C) were also measured under the same conditions as those used for the α-lactalbumin solution measurements. They were subtracted from the α-lactalbumin solution spectra.

1.6. State of OE_2imC_3C solution under subzero temperature

The phase behavior of the 5 wt% OE_2imC_3C solution was investigated using differential scanning calorimetry (DSC, DSC-60A plus, Shimadzu Corporation): cooling to -100 °C at a cooling rate of -1 °C/min followed by heating to 25 °C at a heating rate of 5 °C/min. The proportion of unfrozen part in the 5 wt% OE_2imC_3C solution was estimated from the area of the melting peak at around 0 °C.

2. Supplemental Figures

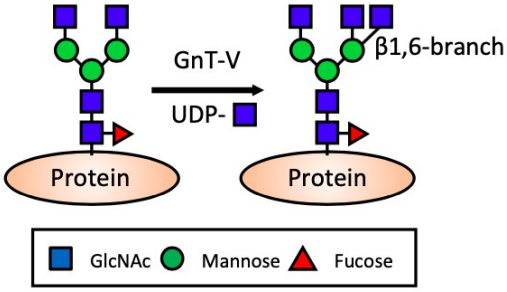

Fig. S1. Schematic image of GnT-V reaction. GnT-V catalyzes the transfer of N-acetylglucosamine (GlcNAc) residue to N-glycan, producing β1,6-GlcNAc branch. Uridine diphosphate (UDP)-GlcNAc is used as a donor substrate.

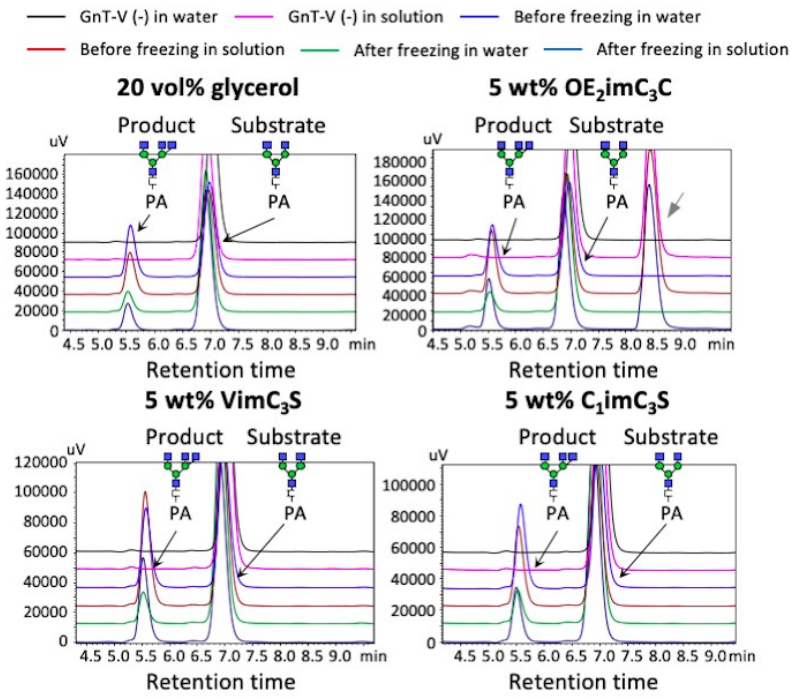

Fig. S2. In vitro activity of GnT-V in the ZI solutions before and after freezing. Purified recombinant GnT-V in water or the indicated solutions before or after freezing at -80℃ was incubated with an acceptor substrate and a donor substrate (UDP-GlcNAc) at 37℃ for 1 h. The product of GnT-V reaction and the unreacted substrate were separated with HPLC. The structures of the substrate and GnT-V product were indicated. A gray arrow indicates the non-specific peak of OE_2imC_3C.

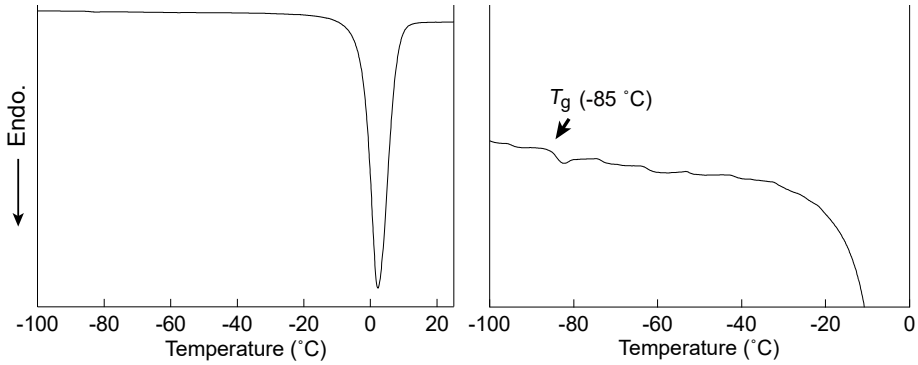

Fig. S3. DSC charts of 5 wt% OE_2imC_3C solution (the arrows indicate the glass transition temperature). The right-hand side chart is the magnified one.

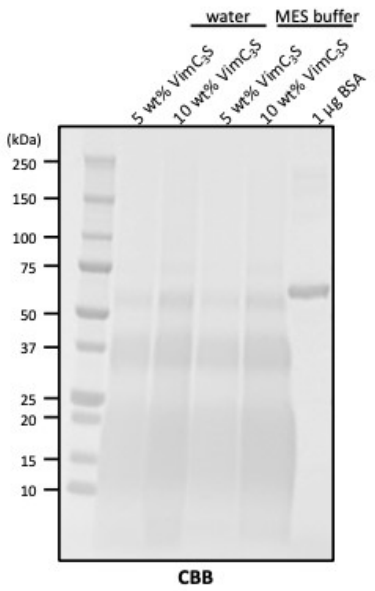

Fig. S4. CBB staining of the protein-free $VimC_3S$ solutions. 5 or 10 wt% of the protein-free $VimC_3S$ solutions in water or 50 mM MES buffer were loaded on SDS-polyacrylamide gel and the gel was stained with CBB.

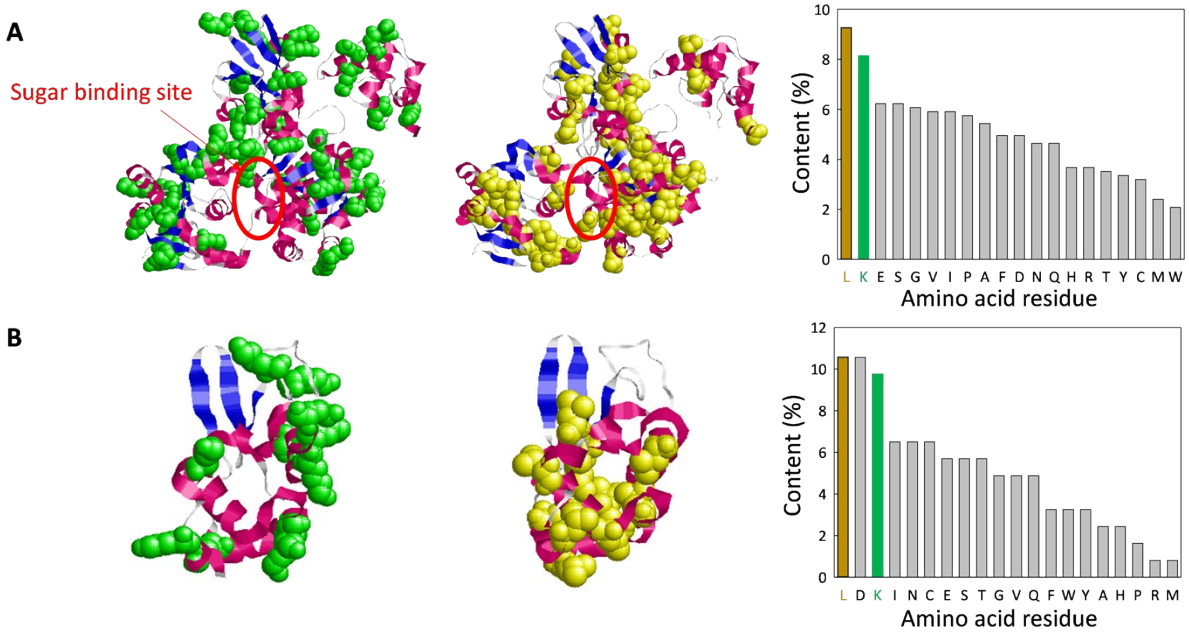

Fig. S5. Three dimensional structures and contents of amino acid residues of human GnT-V and bovine α-lactalbumin (α-Lac). **A.** Structure of human GnT-V (PDB: 5ZIB)[1]. **B.** Structure of α-Lac (PDB: 6IP9)[7]. Green and yellow spacefills display the Lys and Leu residues, respectively.

3. References

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